alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on the anhydrous basis.

(7) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should be developed in the benzene layer.

(8) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.51a Sterile sodium novobiocin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Sodium novobiocin is the crystalline monosodium salt at a kind of novobiocin or a mixture of two or more such salts. It is so purified and dried that:

(i) Its potency is not less than 850 micrograms of novobiocin per milligram, calculated on an anhydrous basis.

(ii) It is sterile.

(iii) It is nonpyrogenic.

(iv) [Reserved]

(v) Its loss on drying is not more than 6.0 percent.

(vi) Its pH in a solution containing 25 milligrams per milliliter is not less than 6.5 and not more than 8.5.

(vii) Its residue on ignition is not less than 10.5 percent and not more than 12.0 percent calculated on an anhydrous basis.

(viii) Its specific rotation in an acidmethyl alcohol solution at 25° C. is not less than −50° and not more than −58°.

(ix) It demonstrates a positive color identity test.

(x) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, sterility, pyrogens, loss on drying, pH, residue on ignition, specific rotation, identity, and crystallinity.

(ii) Samples required:

(*a*) For all tests except sterility: 10 packages, each containing approximately 600 milligrams.

(*b*) For sterility testing: 20 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient 0.1*M* potassium phosphate buffer, pH 8.0 (solution 3), to give a stock solution of convenient concentration. Further dilute with 10 percent potassium phosphate buffer, pH 6.0 (solution 6), to the reference concentration of 0.5 microgram of novobiocin per milliliter (estimated).

(2) *Sterility.* Proceed as directed in § 436.20 of this chapter, using the method described in paragraph (e)(1) of that section.

(3) *Pyrogens.* Proceed as directed in § 436.32(a) of this chapter, using a solution containing 10 milligrams of novobiocin per milliliter.

(4) [Reserved]

(5) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter.

(6) *pH.* Proceed as directed in § 436.202 of this chapter, using a solution containing 25 milligrams of sodium novobiocin per milliliter.

(7) *Residue on ignition.* Proceed as directed in § 436.207(b) of this chapter, calculating on the basis of an anhydrous sample weight.

(8) *Specific rotation.* Accurately weigh approximately 1.25 grams of the sample in a 25-milliliter glass-stoppered volumetric flask. Prepare an acid-methyl alcohol solution by diluting 1.0 milliliter of concentrated hydrochloric acid to a volume of 100 milliliters with absolute methyl alcohol and mix well. Dissolve the sample in about 15-milliliters of the acid-methyl alcohol solution. Adjust to volume with the acid-methyl alcohol solution and mix well. Proceed as directed in § 436.210 of this chapter, using a 2.0-decimeter polarimeter tube. Calculate the specific rotation on an anhydrous basis.

(9) *Identity.* (i) Using 0.1*M* aqueous sodium borate as a diluent, prepare 10 milliliters of a solution containing the equivalent of 1 milligram (approximate) of novobiocin per milliliter.

(ii) Prepare a saturated aqueous solution of *N*,2,6-trichloroquinoneimine by shaking continuously for 30 minutes in a dark bottle 25 milligrams of *N*,2,6-trichloroquinoneimine in 100 milliliters of distilled water. Let stand 2 hours after shaking. Store in the dark bottle.

(iii) Add 2.0 milliliters of the saturated *N*,2,6-trichloroquinoneimine solution to 4 milliliters of the novobiocin solution. Mix well and heat in a water bath at 37° C. for 10 minutes. The development of a blue color is a positive test for the presence of novobiocin. To 2 milliliters of the blue solution, add 2 milliliters of *N*-butyl alcohol and shake well. A green color should develop in the butyl alcohol layer. To the other 2-milliliter portion of the blue solution, add 2 milliliters of benzene (c.p.), and shake well. A pink color should develop in the benzene layer.

(10) *Crystallinity.* Proceed as directed in § 436.203(a) of this chapter.

[39 FR 19166, May 30, 1974, as amended at 50 FR 19921, May 13, 1985]

§ 455.70 Rifampin.

(a) *Requirements for certification*—(1) *Standards of identity, strength, quality, and purity.* Rifampin is a red-brown powder. It is 3-(4-methylpiperazinyliminomethyl) rifamycin SV. It is very slightly soluble in water, soluble in ethyl acetate and in methyl alcohol, and freely soluble in chloroform. It is so purified and dried that:

(i) Its potency is not less than 900 micrograms per milligram.

(ii) [Reserved]

(iii) Its loss on drying is not more than 2 percent.

(iv) Its pH is not less than 4.0 and not more than 6.0 in a 1 percent aqueous suspension.

(v) When calculated on the anhydrous basis, its absorptivity at 475 nanometers is 100±4 percent of that of the rifampin working standard, similarly treated.

(vi) It passes the identity test.

(vii) It is crystalline.

(2) *Labeling.* It shall be labeled in accordance with the requirements of § 432.5(b) of this chapter.

(3) *Requests for certification; samples.* In addition to complying with the requirements of § 431.1 of this chapter, each such request shall contain:

(i) Results of tests and assays on the batch for potency, loss on drying, pH, absorptivity, identity, and crystallinity.

(ii) Samples required: 10 packages, each containing approximately 300 milligrams.

(b) *Tests and methods of assay*—(1) *Potency.* Proceed as directed in § 436.105 of this chapter, preparing the sample for assay as follows: Dissolve an accurately weighed sample in sufficient methyl alcohol to give a stock solution containing 1.0 milligram of rifampin per milliliter (estimated). Further dilute an aliquot of the stock solution with 1 percent potassium phosphate buffer, pH 6.0 (solution 1), to the reference concentration of 5.0 micrograms of rifampin per milliliter (estimated).

(2) [Reserved]

(3) *Loss on drying.* Proceed as directed in § 436.200(b) of this chapter, except dry the sample for 4 hours.

(4) *pH.* Proceed as directed in § 436.202 of this chapter, using a 1 percent aqueous suspension.

(5) *Absorptivity.* Determine the absorbance of the sample and standard